Bottom-up and Top-down Controls on the Microzooplankton Community in the Sargasso Sea

by

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ABSTRACT

Microzooplankton, mainly heterotrophic unicellular eukaryotes (protists), play an important role in the cycling of nutrients and carbon in the sunlit (euphotic) zone of the world's oceans. Few studies have investigated the microzooplankton communities in oligotrophic (low-nutrient) oceans, such as the Sargasso Sea. In this study, I investigate the seasonal and interannual dynamics of the heterotrophic protists, particularly the nanoflagellate, dinoflagellate, and ciliate communities, at the Bermuda Atlantic Time Series site and surrounding areas in the Sargasso Sea. In addition, I test the hypotheses that the community is controlled though bottom-up and top-down processes. To evaluate the bottom-up hypothesis, that the protists are controlled by prey availability, I test whether the protist abundance co-varies with the abundance of potential prey groups. Predation experiments with zooplankton were conducted and analyzed to test top-down control on the protists. I found distinguishable trends in biomass of the different protist groups between years and seasons. Nanoflagellates and dinoflagellates had higher biomass during the summer (28 ± 5 mgC/m^2 and 44 ± 21 mgC/m^2) than during the winter (17 ± 8 mgC/m^2 and 30 ± 11 mgC/m^2). Ciliates displayed the opposite trend with a higher average biomass in the winter (15 ± 9 mgC/m^2) than in summer (5 ± 2 mgC/m^2). In testing my bottom-up hypothesis, I found weak but significant positive grazer/prey relationships that indicate that nanoflagellates graze on picophytoplankton in winter and on the pico-cyanobacterium *Prochlorococcus* in summer. I found evidence that ciliates graze on *Synechococcus* in winter. I found weak but significant negative correlation between dinoflagellates and *Prochlorococcus* in summer. The predation experiments testing the top-down hypothesis did not show a clear top-down control, yet other studies

in the region carried out during our investigation period support predation of the protists by the zooplankton. Overall, my results suggest a combination of bottom-up and top-down controls on these heterotrophic protists, however, further investigation is necessary to reveal the detailed trophic dynamics of these communities.

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INTRODUCTION

The microbial loop is essential for the cycling of carbon and nutrients in the sunlit zone of the world's oceans (Azam et al. 1983). Phytoplankton, bacteria, and zooplankton carry out this element cycling within the microbial loop. Phytoplankton are the primary producers that are consumed by zooplankton. In regions dominated by small phytoplankton (<5 μm), the microzooplankton, particularly phagotrophic protists (unicellular eukaryotes) rather than mesozooplankton are known to graze most of the phytoplankton production (Hlaili et al. 2013). In this food web the smaller heterotrophs' role is to provide a link from the small phytoplankton and bacteria to larger zooplankton. The heterotrophic protists are consumed by larger zooplankton grazers, thus transferring primary production to higher trophic levels (Montagnes et al. 2010). Not all of the primary production is transferred to higher trophic levels, most of the carbon is actually recycled within the microbial loop and some is exported to the deep ocean through the sinking of fecal pellets and aggregates (Richardson and Jackson 2007).

In this study, I focus on three groups of microzooplankton grazers; ciliates, dinoflagellates, and nanoflagellates. Ciliates and dinoflagellates, both phagotrophic protists, make up the majority of the microzooplankton grazer population (i.e. zooplankton less than 200 μm); dinoflagellates are the most abundant group (Dussart 1965; Lessard and Murrell 1996). Ciliates are a group of single-celled phagotrophic protist characterized by specialized organelles known as cilia. These short thin outgrowths are used in a similar fashion as flagella, and function as a primary driver of

locomotion and as a feeding mechanism. Ciliates are diverse and can be autotrophic, heterotrophic, or mixotrophic (Agatha et al. 2007). Dinoflagellates are a group of protists that use two flagella in locomotion. Like ciliates, dinoflagellates have diverse nutritional modes and can be autotrophic, heterotrophic, or mixotrophic. Dinoflagellates are usually the more dominant microzooplankton grazers, sometimes feeding upon cells larger than themselves (Lessard 1991; Neuer and Cowles 1994). The abundance of phytoplankton prey directly influences the abundance of ciliate and dinoflagellate grazers (Agawin et al. 2000) in a bottom-up control scenario.

Nanoflagellates are a group of small (<5 μm) flagellated protists that are primarily bactivorous (also known as marine stramenopiles, MAST; Massana 2011), yet are also known to graze on picophytoplankton (Massana et al. 2006). Thus, for this study, I group them with the rest of the microzooplankton grazers. While originally not thought to be important quantitatively, these nanoflagellates are now known to account for a large percentage (~35%) of heterotrophic flagellates in the oligotrophic and other ocean regions (Massana et al. 2006; Massana 2011). Like the ciliates and dinoflagellates, nanoflagellates play an important role in carbon cycling (Sherr and Sherr 2009). These very small flagellates are influenced by the abundance of bacteria and picophytoplankton, and are known to keep these groups at stable concentrations through grazing. Like ciliates and dinoflagellates, nanoflagellates facilitate the transfer of carbon and other elements to higher trophic levels when they are consumed by larger zooplankton (Massana et al. 2006).

Larger zooplankton (such as copepods or euphausiids) exerts grazing pressure on the micrograzers in a top-down control. Thus, the abundance of the microzooplankton community is influenced by the availability of their prey, which in turn is indirectly influenced by nutrient availability, and predation by zooplankton (Sherr and Sherr 2009; Montagnes et al. 2010).

In this study I investigate the nanoflagellate, dinoflagellate, and ciliate communities in the Sargasso Sea, an oligotrophic region located in the subtropical North Atlantic Gyre at the Bermuda Atlantic Time-series Site (BATS) and surrounding areas. Few studies have quantified the microzooplankton community in the Sargasso Sea (Lessard and Murrell 1998); as the majority has focused on the photosynthetic communities and primary production. My study is novel in that it tries to understand not only the contributors to the microzooplankton community in a comprehensive fashion, but also what influences their distribution in the Sargasso Sea.

The Sargasso Sea is characterized by a phytoplankton bloom period in the late winter/ early spring when convective mixing brings nutrients into the euphotic zone, triggering a bloom (Lomas et al. 2013; Lomas et al. 2009). Subsequent stratification of the water column in summer and fall contribute to the characteristic low nutrient levels that are often below detection (<0.01 μM) in the region (Lipschultz 2002).

During the spring and summer of 2011 and 2012, BATS and other locations around BATS were sampled with the objective to characterize these nano- and

microzooplankton communities and to examine if and how the community changes annually and seasonally. In addition, I tested how bottom-up and top-down controls influence the phagotrophic protists both in the environment and in experimental settings. Through understanding the role of microzooplankton in the trophic dynamics of the region I can gain insight into nutrient cycling in the Sargasso Sea. This study was part of a larger, NSF funded multi-investigator project termed "Trophic BATS" aimed at studying trophic controls on carbon export.

I hypothesize that nanoflagellate, dinoflagellate, and ciliate biomass is related to prey availability through a bottom-up control. I predict based on this hypothesis that the biomass of phagotrophic grazers will increase with increasing prey biomass. Further, I hypothesize that ciliate and dinoflagellate biomass is controlled through zooplankton predation (top-down control). I predict that the micrograzer population will be negatively affected by the introduction of zooplankton predators to the system. Overall the micrograzer population will reflect the bottom-up control by prey but also the top-down control of zooplankton.

METHODS

Field Sampling

Seawater samples were collected on four research cruises in the spring and summer of both 2011 and 2012. On each cruise, the BATS (Bermuda Atlantic Time Series, 31´40˚N, 64´10˚W) station and a mesoscale feature (eddy) was sampled. Each location was sampled twice within 48 hours with the exception of locations AC1, ACe1, and Ce2. Table 1 provides a list of locations sampled. At each location, seawater was collected from the euphotic zone using a rosette carrying 21 12-liter Niskin bottles. Conductivity, temperature, and depth (from sensors, CTD) and chlorophyll fluorescence (from an *in situ* fluorometer) were measured as the rosette descended; water samples were collected at specified depths (20 m, 50 m, 80 m, 100 m) as the rosette ascended.

Initial samples were taken immediately from the Niskin bottles and fixed for microscopy with either a 2.5% acid Lugol's solution or 0.1 ml of 50% glutaraldehyde. The acid Lugol's solution was composed of 20 g potassium iodide, 10 g iodine, 20 mL glacial acetic acid, and 200 mL of DDI water. Additionally, 250-400 ml of seawater was filtered onto a GF/F filter and then extracted in 90% acetone for 24 hours for later analysis of chlorophyll concentration. The chlorophyll samples were analyzed onboard with a TD 700 fluorometer. Abundance of cyanobacteria was determined using flow cytometry and provided by Mike Lomas from the Bermuda Institute of Ocean Sciences (Lomas et al. 2010). Cyanobacteria biomass was calculated using the carbon to cell ratio presented by Lomas and Moran (2011).

Table 1. Sampling locations (BATS, different types of mesoscale eddies) sampled over a 2-year period in the Sargasso Sea. Where not otherwise stated, sampling depths were 20, 50, 80, and 100 m.

Season, Cruise Number	Station	Location	Notes
Winter 2011 AE 1102	AC1	Anticyclonic	No 80 m sample
	ACe1	Anticyclonic Edge	No 80 m sample
	B1a	BATS	No 80 m sample
	B1b	BATS	No 80 m sample
Summer 2011 AE 1118	C2a	Cyclonic	No 80 m sample
	C2b	Cyclonic	
	Ce2	Cyclonic Edge	
	B2a	BATS	
	B2b	BATS	
Winter 2012 AE 1206	C3a	Cyclonic	No 100 m sample
	C3b	Cyclonic	
	B3a	BATS	No 100 m sample
	B3b	BATS	
Summer 2012 AE 1219	AC4a	Anticyclonic	
	AC4b	Anticyclonic	
	ACe4a	Anticyclonic Edge	
	ACe4b	Anticyclonic Edge	
	B4a	BATS	
	B4b	BATS	

Inverted Microscopy

Ciliates were quantified in the samples fixed with Lugol's solution. 100 mL of sample was settled for 48 hours and then counted by the Utermöhl settling chamber method using an Olympus IMT-2 inverted microscope at 40X magnification (Utermöhl 1931). The entire microscope slide was viewed. All ciliates were identified, counted, and measurements of ciliate body length and width (excluding the cilia, and lorica in tintinnids) were taken in micrometers. The ciliates were divided into the following phylogenetic groups: oligotrichids, choreotrichids, tintinnids, haptorids, scuticociliates, and a collection of other ciliates that could not be placed into a specific group (Agatha 2007). Patterns in the oral cilia were used to distinguish between oligotrich and choreotrich ciliates. In choreotrich ciliates, the oral cilia form a complete circle whereas in oligotrich ciliates the cilia are in two rows, one ventral and the other along the girdle. Tintinnid ciliates were easily identified based on their lorica, a shell-like outer covering. Further, tintinnid ciliates can be classified based on their lorica shape (Agatha et al. 2012). Common tintinnids found included: Codonella, Eutintinnus, Parundella, and Salpingacantha. The haptorid ciliates have oral cilia as well as an equatorial kinety belt. Scuticociliates have cilia that completely surround their cell and may also have a caudal cilium present.

These phylogenetic groups were condensed further; tintinnid ciliates were combined with choreotrich ciliates as their abundances (< 30 cells/L) did not warrant a separation of the groups; tintinnid is an order in the subclass choreotrich. The oligotrich ciliate group included all identified oligotrichs including the mixotrophic ciliates of the

genus *Tontonia* sp. that could be distinguished by a large dorsal tail, and *Laboea strobila* that has a large ice cream cone-like shape. Due to low abundances (<30 cells/L) haptorid, scuticociliates, and unknown other ciliates were combined into an "other" category. Additionally, the ciliates were assigned to standard shape categories, such as sphere, cone, prolate spheroid, and "ice cream cone" (cone + half sphere). Biovolume (μm^3 per 100 mL) was calculated according to shape (Hillebrand et al. 1999). The biovolume was converted into carbon based on a ratio of carbon to volume (Putt and Stoecker, 1989).

Epifluorescence Microscopy

The samples fixed with glutaraldehyde was prepared for epifluorescence microscopy by filtering 25 mL of water through a 0.2 µm black polycarbonate filter and then staining the filter with 0.2 mL of a 1% solution of 4', 6-diamino-2-phenylindole (DAPI). The DAPI stains nuclear DNA and fluoresces under UV light excitation allowing photosynthetic and heterotrophic organisms to be distinguished. Heterotrophic dinoflagellates, mixotrophic dinoflagellates, nanoflagellates, and picoeukaryotes were counted utilizing a 100x Plan-NEOFLUAR 100x/1.30 oil objective lens on an epifluorescent microscope. Heterotrophic and mixotrophic dinoflagellates were distinguished by their prolate spheroid shape and dinokaryon (bright nucleus) present under UV light. The dinoflagellates were categorized into three conventional size categories (5-10 µm, 11-15 µm, and 16-20 µm for quantification and biomass determinations. Under the blue light excitation, mixotrophic dinoflagellates can be recognized by their red chloroplasts dispersed along the periphery of the cell. However,

mixotrophs were grouped with the heterotrophic dinoflagellates due to low cell counts and the uncertainly in distinguishing them accurately from heterotrophic forms. Nanoflagellates were counted in one size group (1-5 μm) was identified based on their green fluorescence under blue light and circular shape. The photosynthetic eukaryote group was distinguished by their spherical shape with visible red chloroplasts under blue light and was counted in four size categories (1-2 μm, 2-4 μm, 4-6 μm, and >6 μm).

The cells were counted in stripes across the filter. Counting continued until at least 30 cells from each group were counted in order to achieve a 95% confidence interval of ± 30% of the cell count according to Lund et al. (1958). Biovolume was calculated for each group based on cell size and shape (prolate spheroid or sphere). The biovolume was converted into carbon based on a ratio of carbon to volume (Menden-Deuer and Lessard 2000).

Predation Experiments

During all four cruises multiple zooplankton predation experiments were conducted by another group involved in the "Trophic BATS" project. [Due to error in the experimental set-up and implementation, experiments from the first three cruises were not used in this study.] Predation experiments from summer 2012 were used in this study. On this cruise three zooplankton predation experiments were conducted (Table 2). For each experiment, 200 μm filtered seawater (FSW to exclude mesozooplankton grazers) was collected as an initial sample. The grazing experiment consisted of triplicate controls and treatments in 2L polycarbonate bottles that were incubated in the dark and

kept at ambient temperature using a flow-through seawater system. The control consisted of only 200 µm FSW. Each treatment involved the addition of several individuals of certain groups of mesozooplankton grazers (see Table 2) to the bottles containing 200 µm FSW. Treatments and controls were sampled after 12 hours. Each sample taken was fixed and prepared for inverted and epifluorescene microscopy as described above.

Table 2. Setup of the three different predation experiments conducted on cruise AE 1219 in summer 2012. Control without zooplankton addition and treatments (each n=3) with a community of crustacean zooplankton added were sampled after 12 hours.

Station	Date	Control Samples	Treatment Received	Number Zooplankton	Incubation Time (hr)
AC4a	7/20/2012	3	Calanoid	5	12
ACe4a	7/24/2012	3	Eucalanus	5	12
B4a	7/28/2012	3	Mixed	n/a	12

Statistical Analyses

Multiple analyses were used to test for statistical significance in the data. Comparisons of biomass between years and seasons were made using a paired t-test (p-value <0.05). A one-way analysis of variance (ANOVA) was used to test if sample stations were significantly different from each other. An ANOVA was also used to address changes in the community composition with depth in the water column. Linear regression was used to test the bottom-up control hypothesis that the biomass of prey groups affected the biomass of grazers. Finally, in the predation experiment a paired t-test was used to assess whether microzooplankton populations decreased significantly with the addition of zooplankton predators (top-down).

RESULTS

Hydrography

During the winter, the water column was well-mixed and the mixed-layer depth (MLD) ranged from 85 m to 340 m in 2011 and 65 m to 166 m in 2012. Average water temperature in the winter decreased with depth from 20 °C to 19.7 °C in 2011 and 20.1 °C to 19.7 °C in 2012. During the summer, the water column was thermally stratified (decreased from 26.8 °C to 18.9 °C in 2011 and from 26.6 °C to 20.8 °C in 2012; Table 3). In the summer the MLD was between 7 m and 24 m in 2011 and 17 m and 34 m in 2012 (data not shown.

The location of the deep chlorophyll maximum (DCM) was determined from the fluorescence profile. Across all seasons the DCM was at 80 m [except for winter 2011]. Chlorophyll *a* is frequently used as a proxy for phytoplankton biomass. The lowest chlorophyll *a* concentrations were found at 20 m in the summer and 100 m in the winter (Table 3). Once the DCM was determined, water samples were collected from that depth. In the winter, when the MLD, was deeper, surface chlorophyll *a* tended to be lower than under stratified conditions. During the winter chlorophyll *a* concentrations ranged from 0.14 to 0.28 $\mu g/L^{-1}$. The maximum summer chlorophyll concentrations, 0.38 $\mu g/L^{-1}$, exceeded those found in the winter (Table 3). A paired t-test for the depth resolved averaged chlorophyll concentrations showed no difference between seasons (p-value > 0.05, data not shown).

Table 3. Mean and standard deviation of temperature (°C) and chlorophyll concentration (µg/L^{-1}) for each season and depth.

Season	Depth (m)	T (°C)	Chl (µg/L^{-1})	Season	Depth (m)	T (°C)	Chl (µg/L^{-1})
Winter 2011	20	20.0 ± 0.9	0.21 ± 0.12	Summer 2011	20	26.8 ± 0.5	0.05 ± 0.01
	50	20.0 ± 1	0.22 ± 0.10		50	21.6 ± 0.3	0.12 ± 0.02
	80	*nd	*nd		80	19.3 ± 0.4	0.38 ± 0.14
	100	19.7 ± 0.9	0.21 ± 0.10		100	18.9 ± 0.3	0.28 ± 0.06
Winter 2012	20	20.1 ± 0.6	0.16 ± 0.07	Summer 2012	20	26.6 ± 0.5	0.07 ± 0.02
	50	20.0 ± 0.5	0.25 ± 0.07		50	23.5 ± 1.4	0.11 ± 0.04
	80	19.5 ± 0.3	0.28 ± 0.09		80	21.8 ± 1.4	0.33 ± 0.14
	100	19.7 ± 0.2	0.14 ± 0.08		100	20.8 ± 1	0.30 ± 0.11

*Note: *nd = no data; 80 m was not sampled in winter 2011.*

The micro-zooplankton community

I did not find any significant differences in protist biomass between eddies sampled within any season and year (one-way ANOVA p-value >0.05; Fig. 1). Therefore, I present seasonally integrated biomass data for each protist group. Protist biomass also did not show any significant differences between depth (one-way ANOVA p-value > 0.05; data not shown), thus seasonally integrated data include all depths sampled (Table 3).

The total protist biomass was comparable between seasons and years (Fig. 1). Nanoflagellate biomass fluctuated seasonally with the lowest biomass occurring during the winter of 2011, 10 ± 5 mgC/m^2 (paired t-test, p-value = 0.01). This biomass was significantly lower than during the following winter in 2012 (25 ± 5 mgC/m^2; Table 4). The summer biomass was significantly higher than in the winter (p-value = 0.04). There

was no notable nor significant (p-value > 0.05) trend in the nanoflagellate biomass between the two summers.

Dinoflagellate biomass was at its highest in summer 2011 (169 ± 46 mgC/m^2), significantly higher than in summer 2012 (95 ± 35 mgC/m^2; paired t-test, p-value = 0.05; Fig. 1, Table 4). There was no difference in total biomass between the winter samples. However, dinoflagellates of 5-10 μm in size were the dominant group, except winter 2011 when the 11-15 μm size class was more abundant. The changes in dinoflagellate size distribution were not significantly different between seasons.

Ciliate biomass was highest during the winter 2011 and did not show significant differences between years or seasons (Table 4, paired t-test, p-value > 0.05). The choreotrich ciliates typically had the highest biomass across all seasons with the exception of winter 2011 when haptorid ciliates and a few larger unknown ciliates (> 30 μm) contributed to the higher biomass in winter 2011.

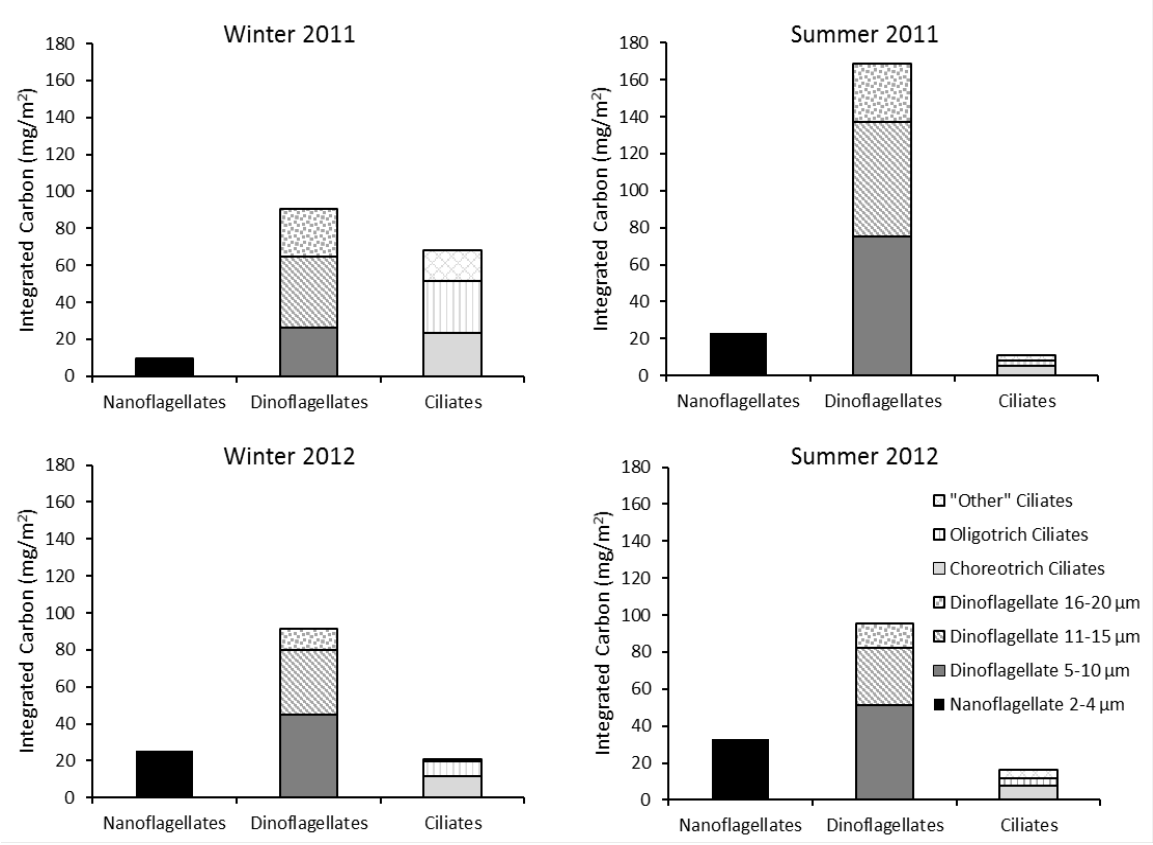

Figure 1. Seasonally integrated biomass (mgC/m^2) of nanoflagellates (2-4 µm), dinoflagellates (5-10 µm, 11-15 µm, 16-20 µm), and ciliates (choreotrich, oligotrich, "other"). The biomass presented is the mean of all stations sampled in the respective season and year (Winter 2011: n=4, Summer 2011: n=5, Winter 2012: n=4, Summer 2012: n=6). For statistics see Table 4.

Table 4. Mean, standard deviation, and p-value (<0.05; paired t-test) for the seasonally integrated biomass (mgC/m^2) for each group presented in Figure 1. A paired t-test was used to test for differences between seasons and years (p < 0.05 in bold).

		Nanoflagellates (mgC/m^2)	Dinoflagellates (mgC/m^2)	Ciliates (mgC/m^2)
Mean ± SD	Winter 2011	10 ± 5	90 ± 60	68 ± 67
	Summer 2011	23 ± 4	169 ± 46	11 ± 6
	Winter 2012	25 ± 5	91 ± 19	24 ± 10
	Summer 2012	33 ± 14	95 ± 35	16 ± 9
p-value	Winter /Summer	**0.05**	0.27	0.19
	Winter 2011/2012	**0.01**	0.98	0.43
	Summer 2011/2012	0.29	**0.05**	0.36

To illustrate the variability that can exist within the protist population at any given site, I investigated seasonal and inter-annual variability at the BATS station (Fig. 2). Samples from 2011 had a higher protist biomass than the samples from 2012. For each season and year, an average biomass for each protist group was calculated from the replicated stations. There was no significant difference between replicate stations (paired t-test, p-value > 0.05), although the integrated biomass at B1a (215 mgC/m^2) was much higher than at B1b (45 mgC/m^2).

While there were no significant interannual or seasonal differences (paired t-test, p-value > 0.05) within the protist groups at the BATS site (Table 5), a few patterns are recognizable. Nanoflagellate biomass (mgC/m^2) was lowest during winter 2011. During the remainder of the sampling period, the mean nanoflagellate biomass was relatively constant, around 27 ± 6 mgC/m^2. Among the dinoflagellates, the 5-10 μm size class was the most dominant in 2012. The 11-15 μm group dominated biomass in summer of 2011 (85 mgC/m^2). In winter 2011, the ciliate biomass peaked and was dominated by the oligotrich and "other" ciliates. Throughout all other seasons the ciliates were relatively consistent in biomass, which ranged from 5 to 12 mgC/m^2 and were dominated by the choreotrich ciliates (with the exception of summer 2012 when the "other" ciliates were the most abundant group; Fig. 2).

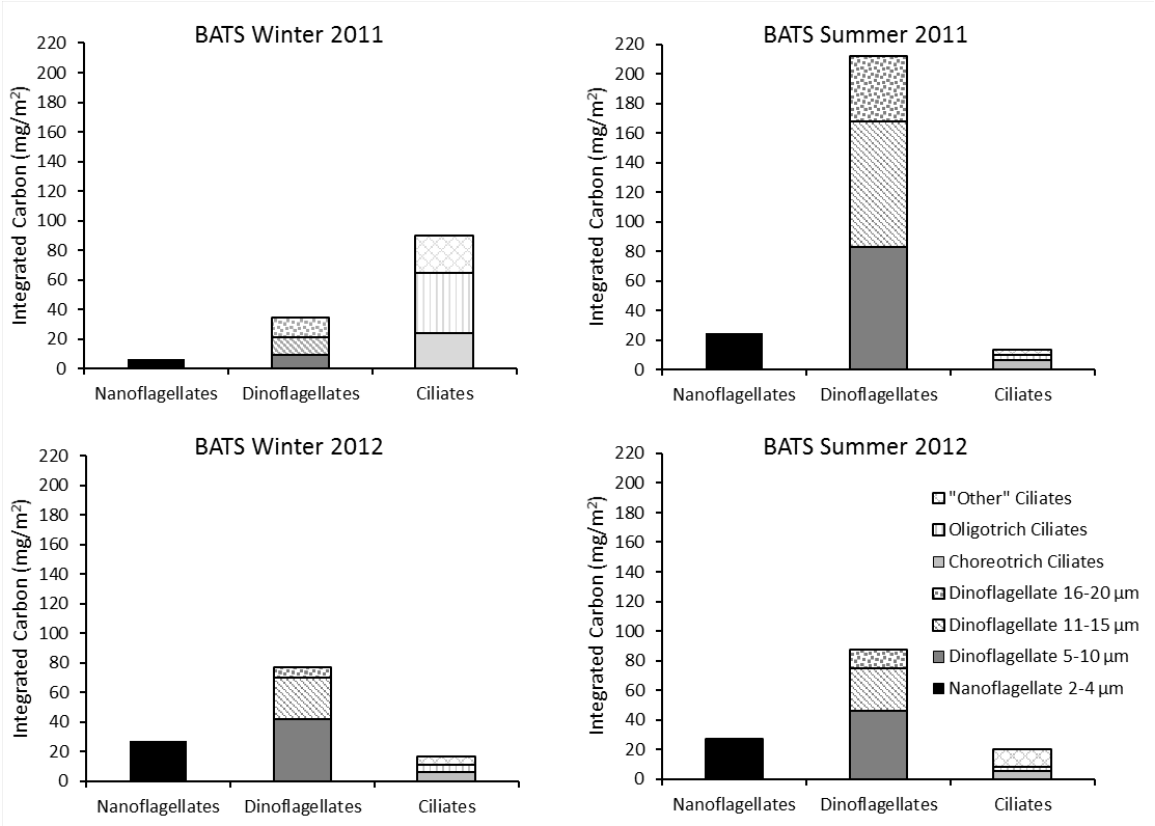

Figure 2. Seasonally integrated biomass (mgC/m^2) of the nanoflagellate (2-4 µm), dinoflagellate (5-10 µm, 11-15 µm, 16-20 µm), and ciliate (choreotrich, oligotrich, "other") groups collected at BATS. Values are the mean of the stations sampled at BATS within the respective season and year. Summer 2011 does not have a replicate for the nanoflagellate and dinoflagellate groups. For statistics see Table 5.

Table 5. Mean, standard deviation, and p-value (< 0.05; paired t-test) for the seasonally integrated heterotrophic protist biomass (mgC/m^2) at BATS. A paired t-test was used to calculate significance of difference between seasons and years.

		Nanoflagellates (mgC/m^2)	Dinoflagellates (mgC/m^2)	Ciliates (mgC/m^2)
Mean + SD	Winter 2011	6 ± 3	34 ± 8	90 ± 74
	Summer 2011	*nd	*nd	13 ± 3
	Winter 2012	27 ± 6	77 ± 18	17 ± 4
	Summer 2012	27 ± 5	87 ± 16	12 ± 6
p-value	Winter/ Summer	0.26	0.22	0.31
	Winter 2011/2012	0.09	0.16	0.41
	Summer 2011/2012	*nd	*nd	0.80

*Note: *nd = no data; Data for summer 2011 are not replicated for the nanoflagellate and dinoflagellate groups, therefore mean, standard deviation, and p-values could not be calculated.*

The ciliates at BATS were grouped into four size classes, 5-10 µm, 10-20 µm, 20-50 µm, and >50 µm (Fig. 3). The most abundant ciliates across all seasons were found in the 10-20 µm size group, and consisted of choreotrich and oligotrich ciliates. In winter 2011 there was a bloom (average of >470 cells/L) of cyclotrich ciliates, primarily the mixotrophic ciliate *Mesodinium rubrum* in both the 10-20 µm and 20-50 µm size groups. *Mesodinium rubrum* was most abundant at 20 m (520 cells/L) and their numbers decreased with depth. *Mesodinium rubrum* was only found during the 2011 winter bloom at the BATS site.

The 20-50 µm ciliate group was dominated by the oligotrich ciliates and was the second most abundant size group (except in summer 2011; Fig. 3). In summer 2011, the 5-10 µm choreotrichs were the second most abundant group. The 10-20 µm and 20-50 µm groups dominated the winter community. The >50 µm group was most abundant during the winter 2011 bloom, and I found *Tontonia*, loricated tintinnid ciliates, and other large unidentified ciliates in this size group. The 5-10 µm group showed no trend, but its contribution to biomass varied among the sampled seasons; this group included mostly choreotrich, scutico-, and a few oligotrich ciliates.

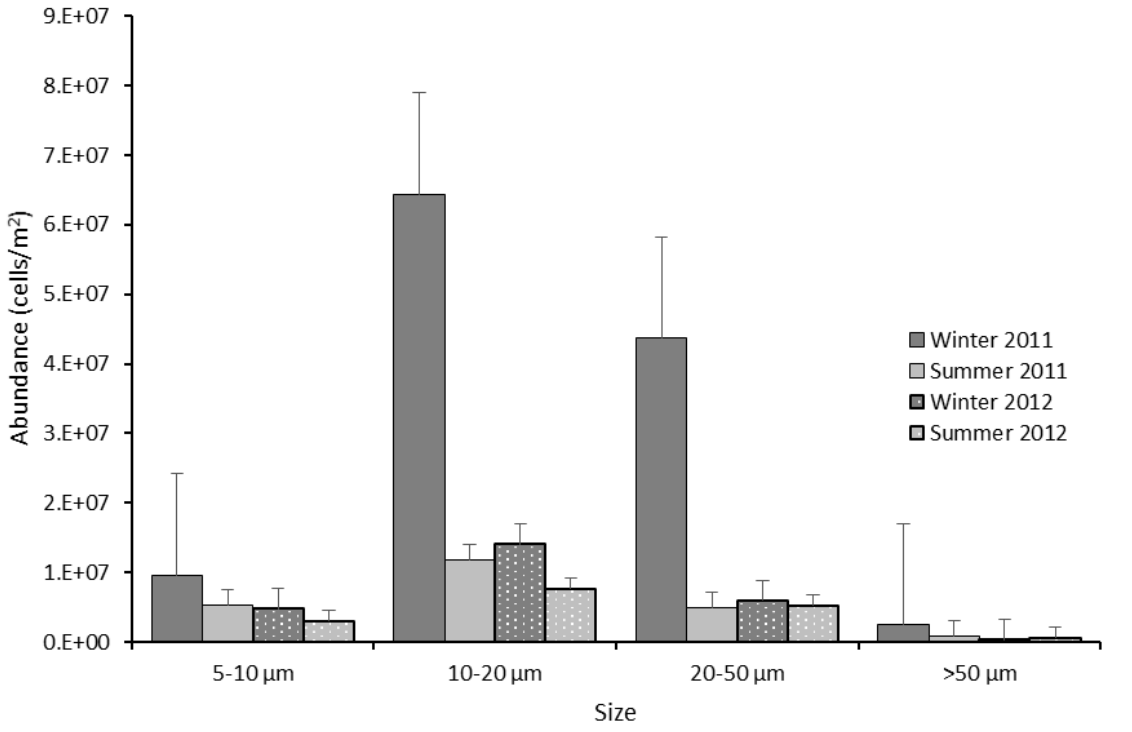

Figure 3. Integrated abundance (cells/m^2) for ciliate size groups (5-10 µm, 10-20 µm, 20-50 µm, and > 50 µm) at BATS for each season. Error bars are one standard deviation around the mean (n=2), each season.

Nanoflagellate biomass was usually highest at 20 m with the exception of winter 2012, where the highest value was at 50 m (Fig. 4). Outside of this data point, nanoflagellate biomass consistently decreased with depth. During the winter of 2012 and in both summers dinoflagellates biomass was highest at 20 m and decreased with depth. In the winter of 2011 dinoflagellates showed the opposite pattern with the lowest biomass at 20 m and increase in biomass with depth. The ciliates (as shown in Fig. 1) biomass was highest in winter 2011; ciliate biomass concentrated at 20 m and decreased with depth. A similar trend occurred in winter of 2012. During the summer seasons, the peak ciliate biomass corresponded with the peak chlorophyll *a* concentration at 80 m (more pronounced in 2011 than 2012). All of the seasonally averaged data analyzed to assess

community composition changes with depth in the euphotic zone (Fig. 4) using a one-way ANOVA. This test was conducted for nanoflagellates, dinoflagellates, and ciliates; and I found no significant differences across depth for these groups.

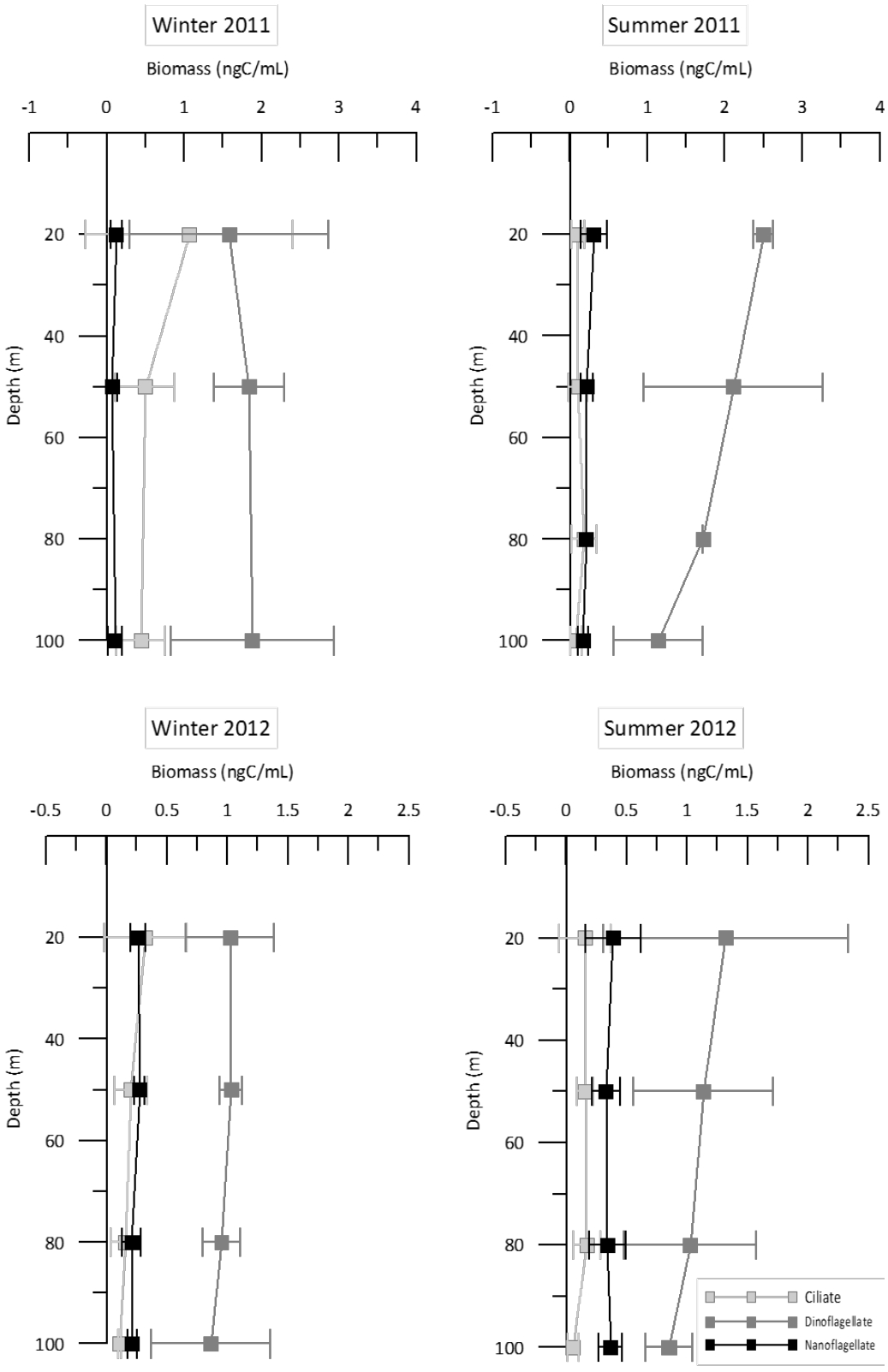

Figure 4. Depth resolved (20, 50, 80, 100 m) biomass (ngC/mL) of the nanoflagellate, dinoflagellate, and ciliate communities. No sample was collected at 80 m during the winter 2011 cruise.

Predator-Prey Relationships

To test my bottom-up control hypothesis, regression analyses were to determine if there was a positive relationship between phagotrophic protist biomass and potential prey biomass (Table 6). Overall, the biomass of nanoflagellates correlated significantly with picoeukaryotes and the cyanobacterium *Prochlorococcus.* The biomass of the ciliates was significantly correlated with the cyanobacterium *Synechococcus*. However, the relationships were not particularly strong ($r^2 = 0.13 – 0.26$) among those groups (Table 6). In the winter, there was a slightly stronger relationship present between nanoflagellates and picoeukaryotes and between ciliates and *Synechococcus* ($r^2 = 0.28$-0.39; Table 6). In the summer seasons, nanoflagellates correlated weakly with picoeukaryotes and *Prochlorococcus.* In contrast to all other relationships found, dinoflagellates correlated negatively with *Prochlorococcus* ($r^2 = 0.11 – 0.19$) (Table 6). The potential predator/prey relationships that had slopes significantly different from zero are shown in Figure 5.

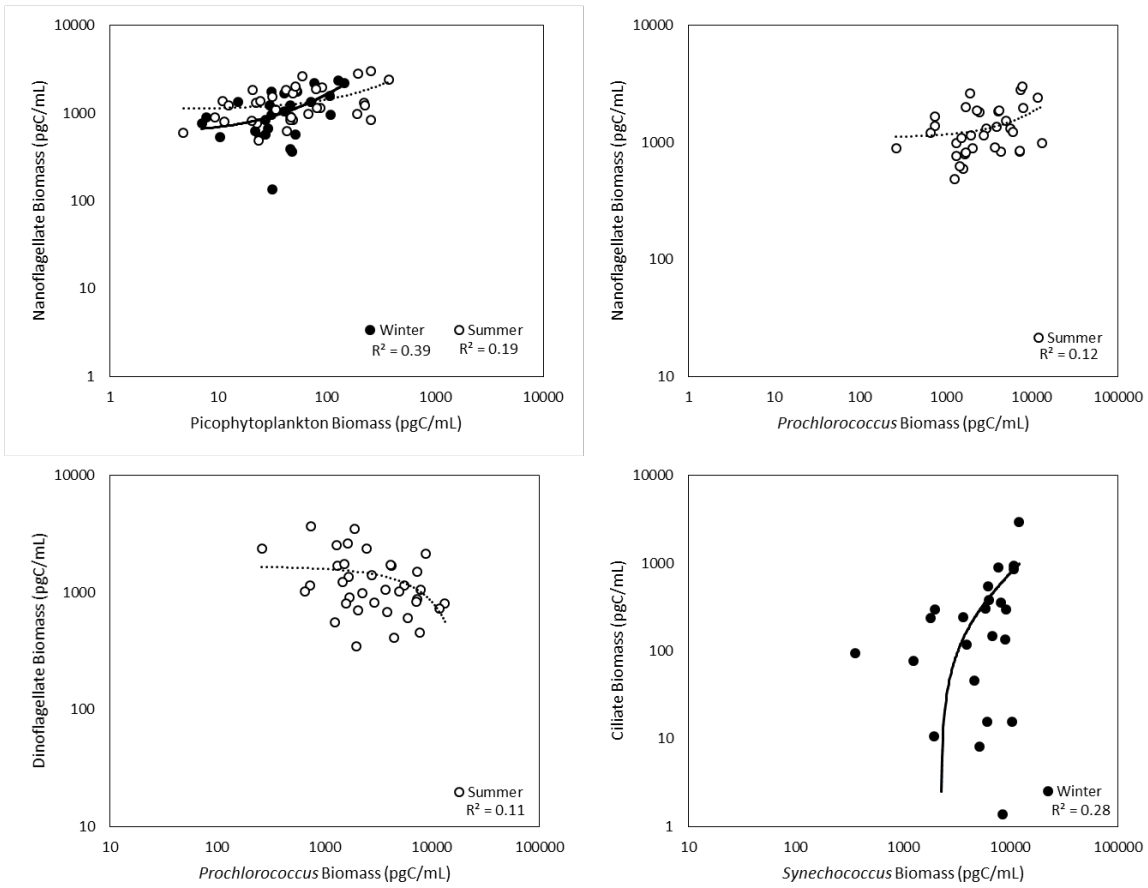

Figure 5. Linear regression of group specific heterotrophic biomass (pgC/mL) plotted against potential prey biomass (pgC/mL) from Table 6. Only groups that have a slope significantly different to zero are graphically shown. Regression lines for summer samples (open circle, dashed line) and winter samples (closed circle, solid line) are shown. Note the log-log axis scale.

Table 6. Linear regression statistics for seasonally combined group-specific heterotrophic protist biomass (pgC/mL) against their potential prey. P-value for the significance of the slope (bold if p< 0.05), and coefficient of determination (r^2) of the regression equation, underlined if slope is significantly different from zero.

		Slope			Slope p-value			r^2		
Season	Prey	Nano-	Dino-	Ciliate	Nano-	Dino-	Ciliate	Nano-	Dino-	Ciliate
All	Nanoflagellates	*nd	+ 0.08	- 0.10	*nd	0.63	0.27	*nd	0.00	0.02
	Picoeukaryotes	+ 4.14	- 2.42	- 0.99	**0**	0.09	0.20	0.23	0.05	0.03
	Synechococcus	- 0.01	+ 0.03	+ 0.06	0.78	0.28	**0.00**	0.00	0.02	0.26
	Prochlorococcus	+ 0.08	- 0.05	- 0.02	**0.01**	0.15	0.39	0.13	0.03	0.01
Winter	Nanoflagellates	*nd	- 0.12	- 0.16	*nd	0.67	0.45	*nd	0.01	0.03
	Picoeukaryotes	+ 10.42	- 5.76	- 4.33	**0.00**	0.21	0.24	0.39	0.06	0.06
	Synechococcus	+ 0.07	+ 0.04	+ 0.10	0.08	0.38	**0.01**	0.13	0.03	0.28
	Prochlorococcus	+ 0.12	+ 0.12	- 0.02	0.25	0.42	0.83	0.05	0.03	0.00
Summer	Nanoflagellates	*nd	+ 0.23	- 0.01	*nd	0.30	0.78	*nd	0.03	0.00
	Picoeukaryotes	+ 3.12	- 2.12	- 0.27	**0.01**	0.18	0.29	0.19	0.05	0.04
	Synechococcus	- 0.13	+ 0.06	- 0.01	0.32	0.30	0.16	0.03	0.03	0.05
	Prochlorococcus	+ 0.07	- 0.08	+ 0	**0.04**	**0.05**	0.48	0.12	0.11	0.01

*Note: *nd=no data*

Predation experiment

Of the three predation experiments conducted in summer 2012, data from only one experiment could be used (Fig. 8A and B) because the other two experiments had zooplankton present in the control samples. A paired t-test was conducted between the initial and control samples to determine if there was significant decline in

microzooplankton biomass during the twelve hour incubation. Another paired t-test was carried out in order to determine if there was significant predation on nanoflagellates, dinoflagellates, and ciliates by larger zooplankton. The predation was calculated by testing the difference in biomass (ngC/mL) between the controls and treatments where the zooplankton predators were added. While the nanoflagellates and the dinoflagellate groups decreased slightly between the controls and treatments, no results were significant (Table 7). I found that while there was a large decrease in biomass between the initial and control sample, the biomass of the choreotrich and "other" groups increased slightly between the control and treatment sample (Fig. 8B).

Mean abundance (cells/L) and biomass (ngC/L) were calculated for the ciliate groups between all three experiments conducted during the summer 2012 cruise (Table 8). The mean excludes any control or treatment samples with copepods present. The abundance and biomass of choreotrich ciliates decreased significantly between the initial and control samples (p-value = 0.05) as well as between the control and treatment samples (non-significant change). The mean biomass of the choreotrich ciliates was greater in the treatment samples than in the controls but this was not statistically significant. The abundance and biomass of the oligotrich and "other" ciliate groups decreased between the initial and control and control and treatment samples, yet those results were not statistically significant.

Predation Experiment #3

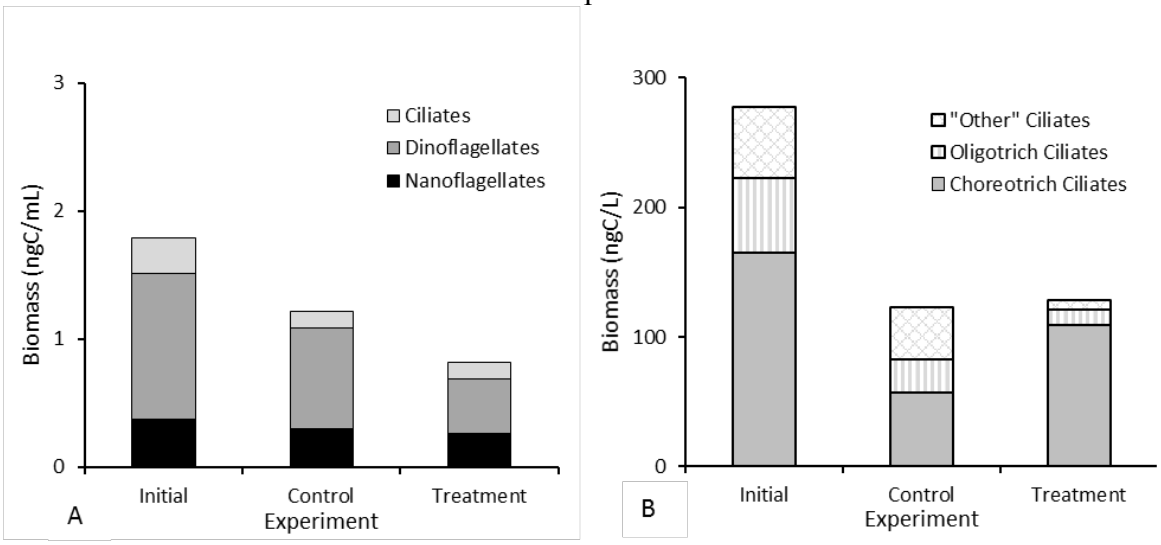

Figure 6. A) Biomass (ngC/mL) of heterotrophic protist groups in the initial (n=1), control (n=3), and treatments (n=3) of the predation experiment. B) As in A) but only for ciliate groups.

Table 7. Mean, standard deviation, and p-values (<0.05; paired t-test) for biomass values in the predation experiment (Figure 6A and B). Paired t-test was used to test for significance of differences between treatment and the control.

Biomass	Mean + SD		p-value
	Control	Treatment	Control / Treatment
ngC/mL			
Synechococcus	182 ± 19	202 ± 10	0.243
Picoeukaryotes	87 ± 66	93 ± 68	0.903
Nanoflagellates	151 ± 151	130 ± 123	0.645
Dinoflagellates	295 ± 115	214 ± 107	0.117
ngC/L			
Choreotrich Ciliates	34 ± 12	65 ± 56	0.670
Oligotrich Ciliates	25 ± 5	12 ± 7	0.664
"Other" Ciliates	213 ± 309	22 ± 0	0.094

Table 8. Mean and standard deviation of abundance (cells/L) and biomass (ngC/L) of predation experiments (n=3) for ciliate subgroups. Paired t-test (p-value <0.05, in bold) was used to test for differences between initial and control samples and between control and treatment samples.

	Mean + SD (cells/L)			p-value (cells/L)		Mean + SD (ngC/L)			p-value (ngC/L)	
	Initial	Control	Treatment	Initial/ Control	Control/ Treatment	Initial	Control	Treatment	Initial / Control	Control / Treatment
Choreotrich Ciliates	78 ± 64	60 ±72	53 ± 44	0.48	0.77	62 ± 50	52 ± 44	93 ± 151	**0.05**	0.39
Oligotrich Ciliates	85 ± 55	36 ± 16	28 ±12	0.30	0.91	60 ± 18	41 ± 85	13 ± 10	0.50	0.32
"Other" Ciliates	20 ± 20	13 ± 5	11 ± 6	0.71	0.73	60 ± 49	97 ± 231	9 ± 7	0.86	0.42

DISCUSSION

My results show that there is a high degree of variability within the protist populations in the Sargasso Sea and at BATS. In most cases, the protist population biomass at BATS and in the surrounding area were not significantly different from each other. However, in two groups I found significant differences which I will discuss below. I also find evidence that bottom-up processes might influence this variability.

Community Trends

Nanoflagellate biomass was significantly greater in the summer than the winter, while dinoflagellate and ciliate biomass remained relatively constant. Additionally, nanoflagellate biomass was significantly higher in winter 2012 than in winter 2011. While the majority of my samples followed the aforementioned trends, the ciliate population at BATS in winter 2011 and dinoflagellate population during the summer 2011 was anomalous. For the ciliates, sampling at B1a revealed bloom-like conditions. Typically, in the North Atlantic, convective mixing of the water column in the winter will bring nutrients into the euphotic zone that allow a bloom of phytoplankton (Lomas et al. 2013; Lipschultz 2002). In the Sargasso Sea, physical forcing such as storms can initiate bloom conditions via nutrient input prior to the water column becoming stratified (Lomas et al. 2009).

At BATS in winter 2011, storms and subsequent stratification of the water column likely helped initiate the bloom of the mixotrophic ciliate *Mesodinium rubrum*. BATS was sampled again two days later during a winter storm (B1b) and the biomass of

the protist populations had decreased, likely because of the greater mixing depth during the storm.

During the bloom at BATS, average ciliate biomass was 1.6 µgC/L, in contrast to an average of 0.15 µgC/L during the other seasons. The subsequent sampling at B1b had an average ciliate biomass of only 0.18µg C/L. While the bloom was mostly comprised of mixotrophic cyclotrich ciliates, other groups experienced an increase in biomass as well. At B1a, a higher concentration of cryptophytes and nanoeukaryotes was also observed (Cotti-Rausch et al. 2015) and the primary productivity at B1a exceeded the productivity on subsequent sampled stations (De Martini 2016).

Outside of the bloom in winter 2011, the protist biomass was comparable to another study in the Sargasso Sea and at BATS (Table 9). Lessard and Murrell (1996) assessed the dinoflagellate and ciliate community in the upper 200 m of the Sargasso Sea during August 1989 and spring 1990. Their ciliate and dinoflagellate biomass data were comparable to the biomass found in my study. This is interesting because it shows that the protist population biomass has remained relatively constant in the twenty-one years between studies. Specifically, in winter/spring the biomass values for ciliates ranged from 0.01-1.4 µgC/L and 0.1-2.3 µgC/L in the summer (Lessard and Murrell, 1996), compared to values between 0.01-2.22 µgC/L in winter and 0.02-0.66 µgC/L in the summer in my study. The dinoflagellate biomass ranged from 0.01-0.09 µgC/L in the summer and 0.01-2.1 µgC/L in the winter/spring (Lessard and Murrell, 1996). The biomass in my study is slightly larger than their reported values in the summer, yet

comparable in the winter/spring. Lessard and Murrell (1996) did not study the nanoflagellate community in the Sargasso Sea.

I also compared my protist biomass values with findings of other studies carried out further north in more productive regions of the North Atlantic. Montagnes et al. (2010) studied ciliate abundances and distribution in the NW Atlantic Ocean. Both Fileman and Leakey (2005) and Stoecker et al. (1994) assessed ciliates and dinoflagellates in the NE Atlantic Ocean. Stoecker et al. (1994) also studied nanoflagellates (Table 9).

Biomass of the nanoflagellates in my study was lower than what was found by Stoecker et al. (1994) during spring 1989 and 1990 in the North Atlantic in the upper 20m (Table 9). Biomass of nanoflagellates in their study ranged from 5.0 – 45.0 µgC/L, whereas in my study the range was 0.02-0.036 µgC/L in the winter and 0.08-0.89 µgC/L in the summer. Stoecker et al. (1994) found a microdiatom bloom in 1989 and a nanodiatom bloom in 1990. The nanodiatoms could have influenced the abundance and size of the phagotrophic protists, resulting in their larger biomass values. The chlorophyll *a* values in the Stoecker et al. study were much higher (0.6 – 3.4 ug/L) than what I found in my study (0.05 – 0.33 ug/L) indicating that potential prey was also much more abundant. In my case, the nanoflagellate biomass during the only "bloom like" conditions I encountered (BATS, winter 2011) had an average value of 0.60 µgC/L which is still much lower than what was found in the Stoecker et al. study.

Additionally, the nanoflagellate biomass was significantly higher in summer than winter, and higher in winter 2012 compared to winter 2011. A study on the trophic coupling of pico- and nano- plankton conducted during the summer 1989 and spring 1990 in the Sargasso Sea found opposite trends (Caron et al. 1999). The abundances and biomass of nanoflagellates was higher in the spring than in the summer, however, there were no significant changes in the populations between the two seasons. Caron et al. (1999) suggests that food limitation by bacteria and picophytoplankton induces the change in nanoflagellate population. Below I will show that picophytoplankton could indeed be the limiting prey item.

Unlike the nanoflagellates, the dinoflagellate biomass in my study was generally comparable to that found in other studies. In the spring of 1989 and 1990 in the North Atlantic, dinoflagellate biomass ranged from 0.01-5.0 and 0.01-15.0 µgC/L, respectively (Stoecker et al. 1994; Table 9). Dinoflagellate biomass in my study was on the lower end of that range; 0.07-2.7 µgC/L in winter and 0.13-3.7 µgC/L in summer. Fileman and Leakey (2005) sampled the upper 20 m of the North Eastern Atlantic in May and June of 1990. During this time they observed the onset of a characteristic North Atlantic spring bloom and dinoflagellate biomass was between 0.32 and 2.24 µgC/L (Table 9), which is very comparable to my data range without bloom conditions. In summer 2011, I found a peak in dinoflagellate biomass of 3.7 µgC/L, the highest biomass value found in my study, and significantly higher than in summer 2012. The increase in dinoflagellate biomass could be attributed to an increase in nanoflagellate prey biomass as the two are positively correlated (Fig. 7; linear regression, r^2=0.45).

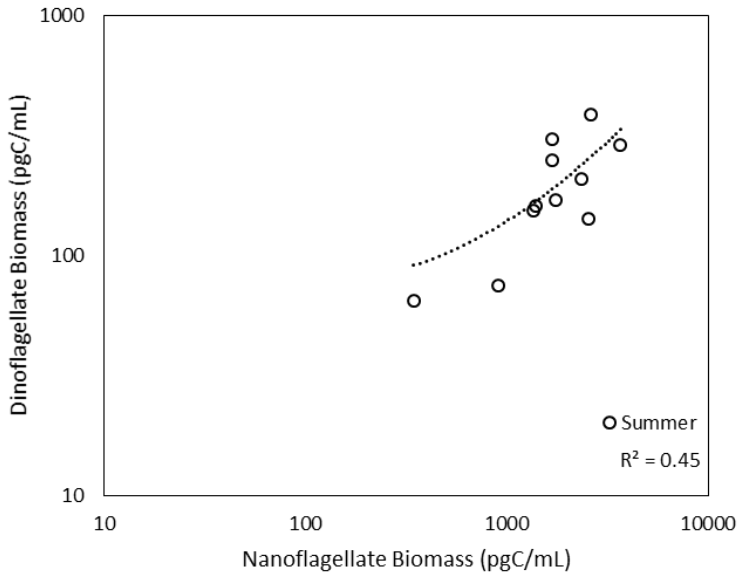

Figure 7. Linear regression of dinoflagellate biomass (pgC/mL) plotted against nanoflagellate biomass (pgC/mL) from Summer 2011. Note the log-log axis scale.

In other studies, ciliate biomass was higher than what I found in my study (Fileman and Leakey 2005; Stoecker et al. 1994). Both Fileman and Leakey (2005) and Stoecker et al. (1994) studied the North Atlantic spring bloom. It is reasonable to expect that ciliate biomass concentrations are higher in bloom conditions. Montagnes et al. (2010) also found much higher ciliate biomass in their study compared to mine; however, the majority of those ciliates (strombidiid) were mixotrophic rather than heterotrophic and might not have had the food limitations that Sargasso Sea ciliates experience (Montangnes et al. 2010).

Table 9. Comparison of results from this study with those from other studies carried out in the North Atlantic Ocean; Montagnes et al. (2010), Fileman and Leakey (2005), Lessard and Murrell (1996), and Stoecker et al. (1994).

Sargasso Sea at and Around BATS				
This Study				
Date	Winter 2011	Summer 2011	Winter 2012	Summer 2012
T °C	19.7 – 20.0	18.9 – 26.8	19.5 – 20.1	20.0 – 26.6
Chlorophyll (ug/L)	0.21 – 0.22	0.05 – 0.38	0.14 – 0.28	0.07 – 0.33
Nanoflagellates (ugC/L)	0.02 – 0.23	0.08 – 0.55	0.10 – 0.36	0.15 – 0.86
Dinoflagellates (ugC/L)	0.21 – 2.72	0.81 – 3.71	0.07 – 1.67	0.13 – 3.35
Ciliates (ugC/L)	0.02 – 2.22	0.02 – 0.38	0.01 – 0.71	0.02 – 0.66
Lessard and Murrell (1996)				
Date	August 1989	March / April 1990		
Chlorophyll (ug/L)	0.03 – 0.4	0.3 – 0.7		
Dinoflagellates ugC/ml	0.1 – 0.9	0.1 – 2.1		
Ciliates ugC/L	0.1 – 1.4	0.1 – 2.3		
Montagnes et al. (2010)				
Irminger Sea (NW Atlantic Ocean)				
Date	Nov / Dec 2001	April / May 2002	July / Aug 2002	
T °C	5.9 – 8.0	5.5 – 8.5	9.6 – 11.0	
Ciliates (ugC/L)	1.0 – 8.0	4.0 – 10.0	8.0 – 32.0	
Fileman and Leakey (2005)				
NE Atlantic Ocean				
Date	May 1990	June 1990		
T °C	12.3 – 14.9	14.5 – 15.6		
Chlorophyll (ug/L)	1.15 – 3.7	0.5 – 2.36		
Dinoflagellates (ugC/L)	0.32 – 1.81	0.5 – 2.24		
Ciliates (ugC/L)	0.84 – 11.06	0.9 – 5.88		
Stoecker et al. (1994)				
NE Atlantic Ocean				
Date	May 1989	May 1-12 1990	May 13-19 1990	June 1990
T °C	13.4 – 13.9	12.3 – 12.8	12.7 – 13.2	14.1 – 15.6
Chlorophyll (ug/L)	2.0 – 4.0	0.6 – 1.9	1.4 – 3.4	0.6 – 1.7
Nanoflagellates (ugC/L)	25.0 – 45.0	9.0 – 17.0	8.0 – 18.0	5.0 – 25.0
Dinoflagellates (ugC/L)	0.01 – 5.0	0.01 – 15.0	0.01 – 12.0	0.05 – 10.0
Ciliates (ugC/L)	2.5 – 11.0	3.0 – 10.0	1.0 – 2.0	0.5 – 2.5

Bottom-up Control

In order to evaluate whether prey availability influenced micrograzer populations (bottom-up control), linear regression analysis was used between grazer biomass and various taxonomic groups of phytoplankton and heterotrophic nanoflagellates that could constitute prey (Table 6, Fig. 5). In most of the cases, the slope was not statistically

different from zero indicating no relationship between predators and their potential prey. In the few cases where a non-zero slope could indicate a bottom-up relationship, the fit to a linear regression line was generally weak ($r^2 = 0.03 – 0.39$). The weak fit suggests that the biomass of the potential prey was not the sole controlling factor on the variability in the micrograzers biomass. Another point to consider is that microzooplankton experience oscillations in their abundance and biomass depending on how much prey is available. The changes in the standing stock of micrograzers are slower than those of faster-growing bacteria and other pico- and nano- plankton (Moloney et al. 1991). It could be that the biomass of micrograzers at the time of sampling are correlated with prey biomass from a few days prior.

When I conducted the linear regression analysis I made a few assumptions regarding the microzooplankton community. I assume in my analysis that the microzooplankton biomass reflects the prey biomass rather than the presence of zooplankton predation. Banse (2013) suggests that a top-down control through predation and grazing is the regulator of phytoplankton biomass which is opposite of what I tested. In this circumstance if predation were controlling the microzooplankton rather than prey availability, a trophic cascade effect would potentially release grazing pressure on the phytoplankton. In addition, I make the assumption that growth rates of both grazer and prey are equal. This assumption can be true for some ocean regions (Strom 2002). Finally, I assume that prey levels are above the threshold food concertation for the grazers and that the microzooplankton were not restricted in their feeding (Lessard and Murrell 1998).

In this study, the nanoflagellates had a stronger correlation with picophytoplankton biomass rather than with cyanobacteria biomass (r^2 = 0.19-0.39, compared to 0.12; Fig. 5). Nanoflagellates are known bacterivores but could be supplementing their diet with picophytoplankton (Sanders et al. 2000). Bacteria concentrations from this study were not available, making speculation about prey availability and selectivity difficult. Li et al. (1992) proposed a ratio of phytoplankton biomass to bacterial biomass in the northern Sargasso Sea in order to test dominance or co-dominance of groups. In their study, cyanobacteria, prochlorophytes, and photosynthetic eukaryotes had the same combined biomass as the bacterial population. This suggests that there was enough bacterial biomass to support the nanoflagellate population. In addition, nanoflagellates exhibit oscillations in their biomass that have been shown to follow bacterial biomass trends (Moloney et al. 1991). When bacterial biomass is lower (due to predation), the nanoflagellates could be consuming other prey items (such as picophyoplankton), which could be reflected as a weak correlation. Nanoflagellates had a stronger correlation with picophytoplankton in the winter than in the summer (Fig. 5).

Dinoflagellates are known grazers of phytoplankton (Sherr and Sherr 2009) but did not correlate with the picophytoplankton; instead showed a weak negative correlation with the cyanobacteria *Prochlorococcus* in the summer (Table 6). The negative trend could be an indication that the *Prochlorococcus* is not being consumed or that their growth is exceeding the increase in dinoflagellate biomass. While there is some statistical correlation, there is too much variability to be able to address any biological

forcing. Other than phytoplankton, high dinoflagellate biomass can be associated with diatom blooms (Strom et al. 2007). Diatoms in the Sargasso Sea are uncommon due to the low biogenic silica concentration except during blooms related (Nelson and Brzezinski 1997) and I encountered very few diatom cells in our samples (< 100 cells/ml). When I used a regression analysis based on all abundance and biomass data (year, season, and depth resolved) for the dinoflagellates and diatoms, I did not find a significant correlation between the two groups (r^2=0.02, abundance; r^2=0.01, biomass).

The only potential prey to which the ciliates showed any correlation were the cyanobacteria of the genus *Synechococcus* (Fig. 5) and this relationship was significant only during the winter. This is understandable since *Synechococcus* is more abundant in the winter than the summer, and ciliates have been shown to have a prey preference for *Synechococcus* over *Prochlorococcus* when presented with both cyanobacteria (Christaki et al. 1999). Like dinoflagellates, ciliates are known to graze primarily on phytoplankton and in some cases on bacterial cells (Hlaili et al. 2013). Determining an exact prey correlation in this circumstance is difficult, as ciliates are known to be selective feeders. Rather than being selective solely based on prey size, ciliates can also be selective of shape and biochemistry of cells (Verity 1991). This selection can vary between individual species of ciliates. I speculate that since ciliates have a variety of feeding modes and potential prey items, it will be difficult to infer a direct correlation between specific prey groups.

Top-down Control

To test the top-down control on the protist population due to zooplankton predators, multiple predation experiments were conducted. The ciliate population was the primary focus of the predation experiment. I predicted, based on the addition of zooplankton predators to the treatment samples that the zooplankton would consume the ciliates and there would be a decrease in overall ciliate biomass in the treatment samples. Additionally, in the control sample, if there was a decline in ciliate biomass between the initial and control, it would be due to cell death rather than zooplankton predation.

The ciliates declined in both abundance and biomass rapidly between the initial sample and the control sample. There was a further, smaller decline between the control and the treatment samples. While that small decline could have been due to zooplankton predation, I was not able to measure if the zooplankton predators actually consumed ciliates and the ciliate decline is more likely due to death of the protists. Ciliates are known to be fragile and do not do well during handling or incubation (Gifford 1985; Stoecker et al. 1994).

In order to support the hypothesis that the ciliate decline was due to cell death and not to predation, the dinoflagellate, nanoflagellate, picoeukaryote, and cyanobacteria communities were examined, as well. All of these groups remained relatively constant in their abundance and biomass between initial, control, and treatment samples, leading me to conclude that no predation was occurring. Nanoflagellates and dinoflagellates (Stoecker and Capuzzo 1990) are an important food source for zooplankton. In this

experiment it is highly unlikely that these two groups were preyed upon, as their abundance and biomass do not reflect a significant decline. However, I was unable to measure zooplankton gut contents or fecal pellets to test if consumption was actually occurring. I also examined the potential prey community of the microzooplankton grazers to assess if they were consuming any prey during the incubation. The populations of picoeukaryotes and cyanobacteria remained relatively constant, indicating that there was negligible consumption taking place.

When I compared my experimental set-up to other zooplankton predation studies conducted with samples from oligotrophic regions, I found distinct differences between the experiments. In two studies from oligotrophic regions, samples were incubated for 24 hours opposed to 12 hours in my study (Perez and Fukai 2007; Broglio et al. 2004). Additionally, the zooplankton were filtered with a smaller mesh size (100 μm) compared to the one used in my study, which could account for zooplankton making it into the initial and control samples. Perez and Fukai (2007) assessed predation on mixotrophic and heterotrophic nanoflagellates and ciliates. They found that predation pressure from copepods was greater for the heterotrophic than for mixotrophic species. Broglio et al. (2004) addressed copepod predation on phytoplankton and ciliates and found that zooplankton selectively fed on ciliates with ciliates providing up to 92.7% (median 37%) of the total carbon intake of copepods.

A concurrent study analyzing zooplankton gut contents and fecal pellets in the Sargasso Sea shows that the protist community was being preyed upon even though those

results were not found in my predation experiment (Wilson et al. in prep). During the study conducted by Wilson et al. (in prep) dinoflagellates were consumed in all four sample periods; predominantly by Pleuromamma spp., and salps in the winter and euphausiids and Pleuromamma spp., in the summer. Ciliates were not found in gut contents or fecal pellets in winter 2011 but were present in summer 2011, winter 2012 (only Pleuromamma spp.), and summer 2012 (euphausiids).

Another study, conducted by De Martini et al. (in prep.) analyzed the plankton community present in the water column and compared it to particulate matter found in sediment traps at 150 m during the same times as my study. De Martini et al. found that ciliates were overrepresented in the sediment trap material in the winter compared to their abundance in the water column. The ciliates were not present in the trap material during the summer. Additionally, dinoflagellates were underrepresented in the trap material compared to the water column. De Martini et al. proposes based on their results that ciliates were actively preyed upon by mesozooplankton during the winter. Although I was not able to quantify the extent of predation on the micro-zooplankton community I speculated that predation was occurring and, to some extent, did exert a top-down control on the protists.

CONCLUSION

The planktonic community in the Sargasso Sea is variable on annual and seasonal time scales. This study was the first study at BATS to analyze annual and seasonal changes for all three phagotrophic protist groups, nanoflagellates, dinoflagellates, and ciliates. Both bottom-up control through prey availability and top-down controls through zooplankton predation likely contribute to the changes in the community at BATS. My results suggest that certain prey (e.g. picophytoplankton for nanoflagellates, the pico-cyanobacterium *Prochlorococcus* for dinoflagellates, and the pico-cyanobacterium *Synechococcus* for ciliates) can influence the microzooplankton community. While analyzing annual and seasonal changes I find there is variability within the biomass of the community. I find that there is more statistical significance in the community changes when examining the surrounding area compared to examining only the BATS site. This is reasonable, as large mesoscale features known as eddies are able to alter the productivity of the ecosystem due to the injection or withdrawal of nutrients. While I did not find differences in my results related to eddy type, they likely contribute to the variability found in the region. In the future, information on nutrient availability, micrograzer community growth rates, and additional predation experiments could help clarify what drives this protist community. Additionally, modeling of the prey/grazer system using collected data such as the ones provided in this study can be equally important in determining trophic relationships beyond what I presented. Through understanding these controls we can further comprehend how trophic interactions shape the plankton community in these oligotrophic regions.

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